Early GalNAc *O*-glycosylation: pushing the tumor boundaries

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ABSTRACT

Glycosylation alterations are frequently observed in cancer cells and shape tumor progression. In this issue of *Cancer Cell*, Nguyen et al. show that GALNT1 relocation from Golgi to endoplasmic reticulum drives liver tumor growth and invasion, due to enhanced glycosylation and consequential activation of the extracellular matrix-degrading metalloproteinase MMP14.

Glycosylation is a complex and finely regulated cellular process that depends on the coordinated action of several glycosyltransferases and glycosidases, exerting a variety of functional roles in physiological and pathological conditions. An highly abundant form of protein posttranslational modification is the GalNAc O-glycosylation, an enzymatic process initiated by a family of up to 20 homologous genes encoding UDP-GalNAc:polypeptide GalNAc-transferases (GALNTs) (EC 2.4.1.41). The GALNTs mediate the GalNAcα1-O-serine / threonine linkage in O-glycoproteins. Although catalyzing the same enzymatic step, each GALNT isoform displays different, though sometimes partly overlapping, acceptor peptide substrate preferences and kinetics features (Bennet et al., 2012). The acceptor peptide specificities comprise both unmodified sequences and previously glycosylated GalNAc-peptide substrates, including the effect of the C-terminal GALNT lectin domain that drives the specificity towards partially glycosylated GalNAc-peptide substrates (Bennett et al., 2012; Revoredo et al., 2016). In addition, GALNT genes display highly controlled cell and tissue expression profiles during development and differentiation. These features are key factors orchestrating the unique cell O-glycoproteome. Classically, GalNAc O-glycosylation of proteins is initiated in the Golgi apparatus after most protein folding events have occurred. In contrast, protein N-Glycosylation and other types of O-glycosylation (including the addition of O-mannose and O-fucose on proteins in the secretory pathway) are initiated in the endoplasmic reticulum (ER) (Bennett et al., 2012).

The cellular glycosylation pattern is recognized to be crucial for several biological functions in normal and disease states. Alterations in enzymes that control different glycosylation steps, resulting in aberrant glycan products, have a clear association with tumor progression, regulating proliferation, invasion and metastasis processes (Pinho and Reis, 2015). Previous studies have reported altered expression of GALNTs during malignant transformation in different types of cancer with association

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with clinicopathological features, such as the presence of venous invasion (Gomes et al., 2009; Pinho and Reis, 2015)

Liver cancer is a major cause of cancer-related death, mainly associated with late diagnosis and frequent metastasis formation. In hepatocellular carcinoma, the most common form of liver cancer, GALNT1 is commonly up-regulated and associated with poor patient survival. Furthermore, its knockdown has been shown to decrease liver cancer cells migration and invasion capacities (Huang et al., 2015). In this issue of Cancer Cell, Nguyen et al. show, besides the striking increase of GALNT1 and of its product, the Tn antigen (GalNAcα1-O-serine / threonine), that the subcellular localization of Tn changes in liver tumor cells. This increased Tn expression was proposed to occur upon the intracellular relocation of GALNT1 from the Golgi to the ER (Nguyen et al., 2017). The mechanism of GALNTs redistribution to the ER has been previously reported to be controlled by the activation of Src tyrosine kinase and COP-I dependent trafficking events (Gill et al., 2010). This mechanism, named the GALA pathway, is based on the relocation of GALNTs leading to increased levels of expression of GALNT mediated O-glycosylation and the Tn antigen expression. ER-location of O-glycosylation has been associated with more aggressive cancer cell features, such as increased migration and invasion (Gill et al., 2013).

Glycans are key players in extracellular matrix (ECM) remodeling. The interactions between cell and ECM are essential for defining an invasive and migratory phenotype (Pinho and Reis, 2015). Using a mouse liver cancer model artificially expressing GALNT1, Nguyen et al. show that the localization of GALNT1 in the ER resulted in increased O-GalNAc glycosylation of various proteins, such as the ER-resident protein PDIA4 and the matrix metalloproteinase-14 (MMP14) (Nguyen et al., 2017). Glycosylation has been previously shown to be important in regulating the activity of matrix metalloproteinases (Boon et al., 2016). Increased activity of the glycosylated MMP14 has been associated with malignant phenotype and metastasis. Nguyen et al. demonstrate an augmented glycosylation of MMP14 produced in cells whose GALNT1 was localized in the ER as compared to Golgi localization, which led to an enhancement in MMP14 activity, potentiating matrix degradation and therefore tumor growth (Nguyen et al., 2017) (Figure 1).

Furthermore, the ER GALNT1 relocation observed in this liver cancer model and concomitant enhanced O-glycosylation was accompanied by a marked increase of circulating tumor cells, which is in line with the high metastatic potential observed (Nguyen et al., 2017).

Overall, the activation of a pathway leading to early O-GalNAc glycosylation of proteins may have a dramatic impact in cancer cell biology. The early GalNAc O-glycosylation in the ER, may favor the action of the lectin domain of other GALNTs and stimulate clustered glycosylation, such as the putative clustered residues in MMP14. The mechanism of tumor progression dependent on increased expression and/or on relocation of GALNT1 may underlie the invasive phenotype associated to metastasis and poor outcome observed in liver cancer (Nguyen et al., 2017; Huang et al., 2015). Given the cell and tissue expression specificity of different GALNTs isoforms it would be of interest to know whether other GALNTs could influence similar mechanisms in different cancer models. Furthermore, the mechanisms leading to GALNTs relocation in tumor cells have generated much interest in the field (Herbomel et al., 2015) and future studies are warranted to fully clarify the molecular events triggering this pathway.

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Noteably, the increased O-glycosylation initiation step through GALNTs may also lead to changes in the biosynthesis of several other cancer relevant glycan structures, such as sialyl-Tn and sialyl-T (Pinho and Reis, 2015). It is therefore expected that the GALA pathway, together with increased expression of other glycosyltransferases, frequently altered in cancers, may generate multiple glycan structures that could impact in the biology of the cancer cells and contribute to the increased aggressiveness of the tumors (Pinho and Reis, 2015).

The study of Nguyen et al. addresses a still not fully clarified mechanism in cancer cells and goes beyond previous studies in unravelling its phenotypic consequences. This work adds new pieces to an intricate network that ultimately results in altered cancer cell glycosylation. The understanding of the regulatory mechanisms underlying these glycosylation modifications, particularly the factors controlling the glycosyltransferases location and activity, hold promise in unraveling novel targets for cancer clinical management.

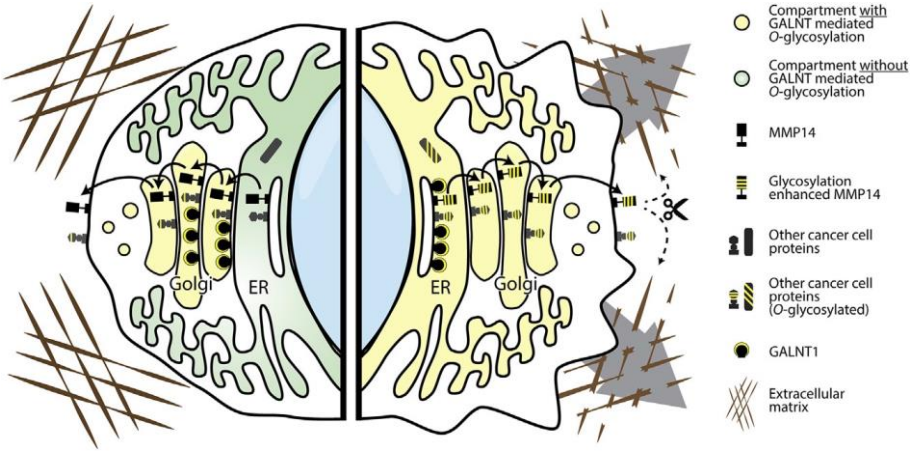

Figure 1. GALNTs subcellular localization affects protein *O*-glycosylation in cancer. Left: Classically, proteins synthesized in the endoplasmic reticulum (ER) undergo the initial step of protein GalNAc *O*-glycosylation in the Golgi apparatus. This step is controlled by GALNTs that lead to GalNAcα1-*O*-serine / threonine linked into *O*-glycoproteins. Several Golgi resident GALNTs catalyze this enzymatic step. The repertoire of GALNTs and other glycosyltransferases expressed define the final glycosylation features of the proteins produced by the cell. Right: Relocation of GALNT1 to the ER can lead to an augmented GalNAc *O*-glycosylation in several proteins expressed by the tumor cell, including the matrix metalloproteinase-14 (MMP14), enhancing its activity, potentiating extracellular matrix degradation and tumor progression.

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REFERENCES

Bennett, E.P., Mandel, U., Clausen, H., Gerken, T.A., Fritz, T.A. and Tabak L.A. (2012). Glycobiology 22, 736-756.

Boon, L., Ugarte-Berzal, E., Vandooren, J. and Opdenakker, G. (2016). Biochem J. 473, 1471-1482.

Gill, D.J., Chia, J., Senewiratne, J. and Bard, F. (2010). J. Cell Biol. 189, 843-858.

Gill, D.J., Tham, K.M., Chia, J., Wang, S.C., Steentoft, C., Clausen, H., Bard-Chapeau, E.A. and Bard, F.A. (2013). Proc Natl Acad Sci U S A. 110, E3152-E3161.

Gomes, J., Marcos, N.T., Berois, N., Osinaga, E., Magalhães, A., Pinto-de-Sousa, J., Almeida, R., Gärtner, F. and Reis, C.A. (2009). J. Histochem. Cytochem. 57, 79-86.

Herbomel, G.G., Rojas, R.E., Tran, D.T., Ajinkya, M., Beck, L. and Tabak, L.A. (2017). PLoS One 12, e0179241.

Huang, M.J., Hu, R.H., Chou, C.H., Hsu, C.L., Liu, Y.W., Huang, J., Hung, J.S., Lai, I.R., Juan, H.F., Yu, S.L., et al. (2015). Oncotarget 6, 5650-5665.

Nguyen, A.T., Chia, J., Ros, M., Hui, K.M., Saltel, F. and Bard F. (2017). Cancer Cell. this issue

Pinho, S.S. and Reis, C.A. (2015). Nat. Rev. Cancer 15, 540-555.

Revoredo, L., Wang, S., Bennett, E.P., Clausen, H., Moremen, K.W., Jarvis, D.L., Ten Hagen, K.G., Tabak, L.A. and Gerken, T.A. (2016). Glycobiology 26, 360-376.

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